Trait means, trait plasticity and trait differences to other species jointly explain species performances in grasslands of varying diversity

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Abstract: Functional traits may help to explain the great variety of species performances in plant communities, but it is not clear whether the magnitude of trait values of a focal species or trait differences to co-occurring species are key for trait-based predictions. In addition, trait expression within species is often plastic, but this variation has been widely neglected in trait-based analyses. We studied functional traits and plant biomass of 59 species in 66 experimental grassland mixtures of varying species richness (Jena Experiment). We related mean species performances (species biomass and relative yield RY) and their plasticities along the diversity gradient to trait-based pedictors involving mean species traits (Tmean), trait plasticities along the diversity gradient (Tslope), extents of trait variation across communities (TCV; coefficient of variation) and hierarchical differences (Tdiff) and trait distances (absolute values of trait differences Tdist) between focal and co-occurring species. Tmean (30–55%) and Tdiff (30–33%) explained most variation in mean species performances and their plasticities, but Tslope (20–25%) was also important in explaining mean species performances. The mean species traits and the trait differences between focal species and neighbors with the greatest explanatory power were related to plant size and stature (shoot length, mass:height ratios) and leaf photosynthetic capacity (specific leaf area, stable carbon isotopes and leaf nitrogen concentration). The contribution of trait plasticities in explaining species performances varied in direction (positive or negative) and involved traits related to photosynthetic capacity, nitrogen acquisition (nitrogen concentrations and stable isotopes) as well as structural stability (shoot carbon concentrations). Our results suggest that incorporating plasticity in trait expression as well as trait differences to co-occurring species is critical for extending trait-based analyses to understand the assembly of plant communities and the contribution of individual species in structuring plant communities.

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Trait means, trait plasticity and trait differences to other species jointly explain species performances in grasslands of varying diversity

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Abstract

Functional traits may help to explain the great variety of species performances in plant communities, but it is not clear whether the magnitude of trait values of a focal species or trait differences to co-occurring species are key for trait-based predictions. In addition, trait expression within species is often plastic, but this variation has been widely neglected in trait-based analyses. We studied functional traits and plant biomass of 59 species in 66 experimental grassland communities of varying species richness (Jena Experiment). We related mean species performances (species biomass and relative yield RY) and their plasticities along the diversity gradient to trait-based pedictors involving mean species traits (T_{mean}), trait plasticities along the diversity gradient (T_{slope}), extents of trait variation across communities (T_{CV}; coefficient of variation) and hierarchical differences (T_{diff}) and trait distances (absolute values of trait differences T_{dist}) between focal and co-occurring species. T_{mean} (30–55%) and T_{diff} (30–33%) explained most variation in mean species performances and their plasticities, but T_{slope} (20–25%) was also important in explaining mean species performances. The mean species traits and the trait differences between focal species and neighbors with the greatest explanatory power were related to plant size and stature (shoot length, mass:height ratios) and leaf photosynthetic capacity (specific leaf area, stable carbon isotopes and leaf nitrogen concentration). The contributions of trait in explaining species performances varied in direction (positive or negative) and involved traits related to photosynthetic capacity, nitrogen acquisition (nitrogen concentrations and stable isotopes) as well as structural stability (shoot carbon concentrations). Our results suggest that incorporating plasticity in trait expression as well as trait differences to co-occurring species is important to understand the varying performances of species in diverse plant communities.

Introduction

In recent years, functional trait-based approaches have been widely adopted to address fundamental questions of community ecology. Functional traits are morphological, physiological or phenological characteristics measurable at the level of individuals, which have an indirect impact on plant performance and fitness via their effects on growth, survival and reproduction (Violle et al. 2007). By being related to the "functioning" of an organism, functional traits may help to understand the mechanisms that determine the coexistence of many species in a community and their varying performances.

The mean expression of functional traits as a general characteristic of a species underlies evolutionary, genetic or physiological constraints and makes the variation among individuals of the same species smaller than variation among species (Suding et al. 2003). Mean trait values are thus the basis of relationships between functional traits and species performance. However, within species, the expression of functional traits may vary due to phenotypic plasticity or genetic variation. Plastic responses of plants might be induced by variation in the abiotic environment or their biotic surrounding, such as varying identities and diversity of plant neighbors (Burns and Strauss 2012, Abakumova et al. 2016, Bennett et al. 2016). In general, high phenotypic plasticity is regarded to confer species superiority in competition (Callaway et al. 2003), but phenotypic responses may also be the result of a reduction in growth due to environmental constraints such as resource limitation (Valladares et al. 2007). Studies in experimental grasslands communities have shown that the expression of functional traits changes in response to increasing species richness, but that species differ in their responsiveness to increased diversity (Gubsch et al. 2011a, Roscher et al. 2011, Lipowsky et al. 2015). Thus, while the direction of trait plasticity in response to increasing species richness is often similar among species, the extents of plastic responses vary greatly between species and between the traits being considered, thereby increasing or decreasing trait similarities among co-occurring species (Lipowsky et al. 2015, Roscher et al. 2015). As a consequence,

trait plasticities may increase or decrease interactions between species in mixtures compared with predictions based traits measurments done in monocultures. Furthermore, varation in trait expression within species across communities is commonly ignored when only mean traits and trait plasticities in response to species richness are used as predictors for plant functioning.

It is often assumed that coexistence between co-occurring species is promoted by trait differences, i.e., species with similar traits tend to compete more strongly and are less likely to locally coexist ("trait difference hypothesis"; Mayfield and Levine 2010, Kraft et al. 2014). According to this hypothesis, the intensity of competition is expected to decrease and niche differences are expected to increase as the absolute values in trait differences among the involved species increases. However, it also has been argued that trait values reflect average fitness differences between species and indicate the position of a species along a competitive hierarchy, i.e., species differences in the ability to exploit the same resource (Herben and Goldberg 2014). According to this hypothesis, both the magnitude as well as the direction of trait differences between the competitors are important predictors of the competitive outcome ("trait hierarchy hypothesis"; Kunstler et al. 2012, Kraft et al. 2014).

So far, it has never been evaluated to which extent different trait-based metrics such as mean species traits, trait plasticities in response to increasing species richness, trait variation within species across communities as well as the disparity of a species from other species explain differences in the average performance of plant species in a plant community and thus species richness effects on community performance such as primary productivity. Here, we present results based on trait and species biomass data of 59 grassland species studied in monocultures and 66 mixtures of varying species richness (2, 4, 8, 16 and 60 species) in a grassland biodiversity field experiment (Jena Experiment; Roscher et al. 2004). Based on the biomass recorded in monoculture and mixture, we derived for each species the average performance in mixture and the performance plasticity in response to increasing species

richness. At he same time we measured mean species traits (Fig. 1a), trait plasticies in response to increasing species richness (Fig. 1b), extents of trait variation across communities (coefficient of variation; Fig. 1c) as well as directional and absolute trait differences between the focal and co-occurring species (Fig. 1d-f). Since competition for resources is an important driver of plant community species composition, we calculated these trait-based metrics using seven traits supposed to be related to the acquisition of light and nutrients as major resources limiting plant growth in temperate grasslands. We used the different metrics to quantify the relations between single or multiple traits and the average performance or performance plasticity in response to increasing plant diversity of species.

Material and methods

Experimental design

The Jena Experiment (Roscher et al. 2004) is located in the floodplain of the river Saale close to the city of Jena (Germany, 50°55`N, 11°35`E, 130 m a.s.l.) on a former arable field. The soil is a Eutric Fluvisol developed from up to 2 m thick fluvial sediments. Because of the fluvial dynamics, the texture ranges from sandy loam near the river to silty clay with increasing distance from the river. The area around Jena has a mean annual air temperature of 9.9°C, and the annual sum of precipitation is 610 mm (1980-2010; Hoffmann et al. 2014). Sixty species typically occurring in Central European mesophilic grasslands (Arrhenatherion type; Ellenberg 1988) were chosen as a species pool for the Jena Experiment. A matrix of morphological, phenological and physiological species characteristics was compiled from the literature to categorize these species into four functional groups: 16 grasses, 12 legumes, 12 small herbs, and 20 tall herbs. The main experiment consists of 78 large plots (20 × 20 m size), which represent all possible combinations of a gradient in species richness (1, 2, 4, 8, and 16 species), crossed near-orthogonally with different levels of functional group richness

(1, 2, 3, and 4 functional groups). Each species richness level had 16 replicates with the exception of only 14 replicates for the 16-species-mixtures (because not enough species were available to create monofunctional legume or small-herb communities with 16 species). Mixtures were assembled by random draws with replacement from the respective functional groups. In addition, 120 smaller plots (3.5 × 3.5 m size) were established to grow all species in replicated monocultures. One replicate per species was abandoned in 2008. Each of four blocks of the Jena Experiment contains an equal number of large plots per species richness × functional group number combination and an equal number of small monocultures per functional group. Plots were established by sowing in May 2002. Total sowing density was 1000 viable seeds per m^2 (adjusted for germination rates from laboratory tests) equally distributed among species in mixture. The sown species richness gradient was maintained by weeding unsown species twice a year (April, July). Plots were not fertilized and mown twice a year (early June, September) according to the typical low-intensity management of unfertilized hay meadows of the Arrhenatherion type. The mown biomass was removed from the field.

Data collection

Species biomass production

Our analysis of species performances is based on data collected at estimated peak biomass before first mowing in late May from 2005 to 2009. Aboveground biomass was harvested 3 cm above the soil surface in randomly placed rectangles of 0.5 × 0.2. Three (May 2005, 2008 and 2009) to four (2006, 2007) or two replicated rectangles were clipped in large plots or small monocultures, respectively. All samples were sorted by species sown in the particular plot; detached dead material was removed. Plant material was dried at 70°C (48 h) and

149 weighed. Replicated samples per plot and species were averaged to estimate species biomass
150 (g_{dw} m^{-2}) for all plots.
151
152 ***Trait data***
153 Plant modules (= single shoots) served as the basic unit for trait measurements. Because most
154 of our species were perennials, a reliable distinction of initially sown genetic individuals
155 (genets according to Harper 1977) was not possible. Measurements were done in one or two
156 monocultures per species, in all mixture plots with 2 to 16 species, and in two replicates of the
157 60-species mixture in late May 2006, with the exception of six species. These six species
158 were studied in May 2008. Transects were installed at a right angle to the plot margin
159 (excluding the outer 70 cm), and every 50 cm (or 25 cm in small plots) one module per
160 species rooting closest to the transects was cut-off at ground level. Three vegetative and three
161 reproductive modules were harvested, when both life stages were present. Five modules were
162 sampled, when only vegetative or reproductive modules were available. Samples were stored
163 in sealed plastic bags in a cool box to prevent dehydration until further processing in the
164 laboratory. There, maximum shoot length was determined. One to five fully expanded leaves
165 from the upper shoot part (dependent on leaf size and number) were taken from each module
166 to measure leaf area with a leaf area meter (LI-3100 Area Meter, LI-COR, Lincoln, USA).
167 Measured leaves and residual plant material were dried at 70°C (48 h) before weighing.
168 Afterwards, samples of measured leaves and all other biomass fractions were pooled per
169 species (separately for vegetative and reproductive modules) and ground with a ball mill.
170 Nitrogen and carbon isotope ratios ($\delta^{15}N$ and $\delta^{13}C$, respectively) as well as nitrogen and
171 carbon concentrations were determined from leaf samples with an isotope-ratio mass
172 spectrometer (IRMS, Delta ^{plus} XP and Delta C prototype respectively, Finnigan MAT,
173 Bremen, Germany). Bulk samples of residual shoot material were analysed for carbon and
174 nitrogen concentrations with an elemental analyzer (Vario EL Element Analyzer, Elementar,

175 Hanau, Germany). Plant traits derived from these measurements are summarized in Table 1.
176 Shoot length (L_{Shoot}), mass height ratios (MHR) and shoot carbon concentrations (C_{Shoot}) are
177 related to space filling and allocation into structural stability. Specific leaf area (SLA) and leaf
178 stable carbon isotopes ($\delta^{13}C$) correlate with photosynthetic capacity and sensitively respond to
179 changes in light and nitrogen availability (Dawson et al. 2002, Hodgson et al. 2011). As traits
180 related to nitrogen acquisition and use, we explored nitrogen concentrations in leaves (N_{Leaf}),
181 which were highly correlated with nitrogen concentrations in the shoots, as well as leaf stable
182 nitrogen isotopes ($\delta^{15}N_{Leaf}$), which depict differences in the uptake of different N forms or
183 facilitation of non-legumes through the provision of legume N derived from N_2 fixation
184 (Högberg 1997). Data were averaged per species and plot to obtain population-level trait
185 values.
186
187 **Data analyses**
188 Population-level aboveground biomass in the mixtures (BM; $g_{dw}\ m^{-2}$), multiplied with species
189 richness to correct for sowing proportion, was considered as a measure of species
190 performance in absolute terms. A relative measure of performance in mixtures, relative yield
191 (RY), was obtained by dividing the population-level performance in mixture through the
192 performance in monocultures for each species in each mixture. This unitless measure
193 corresponds to the Relative Yield as introduced by de Wit and van den Bergh (1965). Values
194 of $RY > 1$ indicate that a species produces more biomass in mixture than expected from
195 monoculture, while values of $RY < 1$ suggest that a species performs relatively worse in
196 mixture than in monoculture. Population-level mean values of BM and RY across study years
197 (2005–2009) were averaged across across mixtures to get a mean estimate of the performance
198 of each species in the mixtures (BM_{mean}, RY_{mean}). Slopes of linear regressions of z-
199 transformed values of RY against the logarithm of sown species richness were computed for
200 each species as measures of each species' performance plasticity in response to the sown plant

diversity gradient (RY_{slope}). One out of the 60 sown species in the Jena Experiment was excluded from these analyses because its monocultures did not produce any biomass in the study years, and thus RYs could not be calculated (*Cardamine pratensis*). Population-level species performances (BM_{mean}, RY_{mean}) as well as performance plasticities (RY_{slope}) were significantly correlated between study years and the mean across study years (Table A2).

The following predictors were derived for each species from trait measurements to explore their effects on species performances and plasticities:

(i) *Trait means* (T_{mean}) were calculated as the mean of population-level trait values in mixtures.

(ii) *Trait plasticities* (T_{slope}) were calculated as the regression slope of z-transformed population-level trait values against the logarithm of sown species richness (including monocultures). This measure is related to the reaction norm, i.e. the change in trait expression in response to an environmental gradient, as an important aspect of plasticity (e.g. Schmid 1992, Valladares et al. 2007).

(iii) *Trait variations* (T_{CV}) were calculated as the coefficient of variation (CV; ratio of standard deviation and mean) of the population-level trait values across mixtures. This measure directly quantifies the amount of trait variation (e.g. Mitchell and Bakker 2014) present across the studied populations of each species.

(iv) *Trait differences* (T_{diff}) calculated as the difference between the population-level trait value of a species taken as focal species (T_{focal}) and the population-level trait values of all co-occurring species ($T_{co\text{-}occurring}$) in a particular mixture:

$$T_{diff} = T_{focal} - T_{co-occurring} \quad \text{(eqn. 1).}$$

Note that trait differences can be positive or negative; this relates to the so-called "trait-hierarchy hypothesis" of Kraft et al. (2014). From this we distinguish the absolute values of the trait differences as follows.

(v) *Absolute trait distances* (T_{dist}):

227 $$T_{dist} = \left| T_{focal} - T_{co-occurring} \right| \quad \text{(eqn. 2).}$$

228 These absolute trait distances relate to the so-called "trait-difference hypothesis" of Kraft et
229 al. (2014). The original application of these measures refers to species pairs (Kraft et al.
230 2014). In our study, we calculated first all possible pairwise differences (distances) between a
231 focal species and each co-occurring species in a community and took the mean to get a
232 community-level value for the focal species. Afterwards, we derived mean differences
233 (distances) for each focal species as the average across all communities, where a focal species
234 occurred.
235 First, each group of trait-based metrics (T_{mean}, T_{slope}, T_{CV}, T_{diff}, T_{dist}) was used separately to
236 predict species performances (BM_{mean}, RY_{mean}) and plasticities (RY_{slope}). Second, the trait-
237 based metrics were used in combination. The function *leaps* in the R library *leaps* (Lumley
238 2009) of the statistical software R3.2.3 (R Development Core Team, http://www.R-
239 project.org) was used to search for the best subset of predictor variables applying the Akaike
240 Information Criterion (AIC) for model selection. The coefficient of determination R^2 is given
241 as a summary measure for explained variation. The function *calc.relimp* in the R library
242 *relaimpo* (Grömping 2006) was used to calculate the relative importance of the different trait-
243 based metrics as R^2 partitioned by averaging over orders among regressors (Lindeman et al.
244 1980). If necessary, data were log-transformed (BM_{mean}, RY_{mean}, T_{mean} in MHR and N_{Leaf}, T_{CV}
245 in all variables except SLA and N_{Leaf}, and T_{dist} in all variables) to meet the assumptions of
246 statistical analyses. Afterwards, predictor variables were z-transformed. Since several
247 predictor variables were correlated (Supplementary Material Appendix Table A3, Fig. A2,
248 A3), we always present the best five models (Tables 2 and A4 for each group of trait-based
249 metrics, and Table 3 for the combination of different metrics), allowing readers to assess the
250 robustness of our analyses. In the results, we refer to those predictors, which were included at
251 least in three out of the best five models.
252

253 ## Results

254

255 **Average species performances and plasticities**

256 The relative performance of species in mixture plots (RY_{mean}) and the plastic response of
257 species performances to the plant diversity gradient (RY_{slope}) were positively related to
258 population-level species biomass (BM_{mean}) in mixture plots (Fig. 2a, b). Overall, 46 out of 59
259 studied species had $RY_{mean} > 1$ indicating that they produced more biomass in mixture than
260 expected from their monoculture biomass (Fig. A1). The plasticity of species biomass
261 (RY_{slope}) correlated positively with RY_{mean} (Fig. 2c), i.e. species that increased their relative
262 yield with increasing plant diversity also had higher average overyielding. Of the 59 species
263 analysed, 34 increased their relative performance with increasing plant diversity (RY_{slope}
264 positive).

265

266 **Predicting species performances in mixtures from species mean traits**

267 Separate analyses of trait-based metrics showed that trait means (T_{mean}) explained the highest
268 proportions of variation (about 22–32%) in population-level species performances (BM_{mean},
269 RY_{mean}) and their plasticities in response to the plant diversity gradient (RY_{slope}; Table 2).
270 Leaf nitrogen concentration (N_{Leaf}) was the best single predictor for species performance, but
271 the combination of several traits improved the model. L_{Shoot}, MHR, N_{Leaf} (all positive effects)
272 and C_{Shoot} (negative effects) were incorporated in the model best explaining BM_{mean} (see
273 Table A4). The model best explaining RY_{mean} contained C_{Shoot} (negative effects) and SLA in
274 addition to N_{Leaf} (both positive effects). For RY_{slope} the best predictor combinations based on
275 T_{mean} mostly included L_{Shoot}, MHR and SLA in addition to N_{Leaf} (all positive effects).

276

277 **Predicting species performances in mixtures from trait plasticities**

278 Trait plasticities (T_{slope}) explained about 27% of the variation in absolute population-level

279 species biomass (BM_{mean}) and about 10–12% of variation in mean relative yields (RY_{mean}) and
280 its plasticities (RY_{slope}), indicating a closer relationship between absolute species
281 performances and trait plasticities than between overyielding and trait plasticities. The
282 combination of trait plasticities that best explained BM_{mean} always comprised MHR, $\delta^{13}C_{Leaf}$
283 (both positive effects) and C_{Shoot} (negative effects), while plasticity in L_{Shoot} (positive effects)
284 best explained RY_{mean}. The combination of trait plasticities that best explained performance
285 plasticities (RY_{slope}) was less consistent (see Table A4).
286
287 **Predicting species performances in mixtures from trait variations**
288 Trait variation (T_{CV}) explained 11% of variation in absolute population-level species biomass
289 (BM_{mean}), but only about 5 and 8% of variation in mean relative yields (RY_{mean}) and its
290 plasticities (RY_{slope}). The models that best explained BM_{mean} and RY_{slope} always comprised
291 variation in MHR (negative effects), while no consistent effects of variation in particular traits
292 were found for RY_{mean} (see Table A4).
293
294 **Predicting species performances in mixtures from their trait differences to other species**
295 Trait differences to co-occurring species (T_{diff}) explained about 28% (and 22%) of the
296 variation in population-level species performances (BM_{mean}, RY_{mean}) and 12% of the variation
297 in RY_{slope} (Table 2). The T_{diff} models best explaining BM_{mean} and RY_{mean} included positive
298 effects of N_{Leaf} and negative effects of C_{Shoot}. In addition, L_{Shoot} and $\delta^{13}C_{Leaf}$ (positive effects)
299 were included in the model for BM_{mean}, and SLA was included in the models for RY_{mean}.
300 Positive effects of L_{Shoot} were also consistent in the models for RY_{Slope}.
301
302 **Predicting species performances in mixtures from their absolute trait distances to other**
303 **species**
304 Absolute trait distances to co-occurring species (T_{dist}) explained small proportions of variation

in species performances and diversity effects on it (4–5%). L_{Shoot} (positive effects) was always included as predictor in the best T_{dist} models for BM_{mean}, while predictors for variation in RY_{mean} and RY_{slope} were less consistent (Tables 2, A4).

Combining different trait-based metrics to predict species performances

The combination of all metrics improved model predictions and increased the proportion of explained variation to 50% for population-level species biomass (BM_{mean}), to 37% for species relative yields (RY_{mean}) and to 48% for their plasticityies (RY_{slope}) (Table 3). The models best explaining species performances always combined predictors based on different metrics. The relative importance of different metrics expressed as R^2 contribution was always largest for trait means (T_{mean}) and trait differences (T_{diff}), which had a similar relative importance in models for BM_{mean} (both 0.30) and RY_{mean} (0.37 and 0.33, respectively). In models for RY_{slope}, the relative importance of T_{mean} (0.55) was greater than of T_{diff} (0.33) (Fig. 3). Trait plasticities (T_{slope}) had slightly lower relative importances in models for BM_{mean} and RY_{slope} (0.25 and 0.22, respectively), but were negligible in models of RY_{slope} (relative importance 0.03). Trait variation (T_{CV}) had a relative importance of 0.14 in models of BM_{mean}, while its relative importance did not exceed values of 0.05 in models for relative yields (RY_{mean}, RY_{slope}). Trait distances (T_{dist}) were least important in explaining variation in species performances.

The combined models showed that population-level species biomass (BM_{mean}) was larger in species with greater means in L_{Shoot} and MHR (Table 3). In addition, the combined model best explaining variation in BM_{mean} included $\delta^{13}C_{Leaf}$ and $\delta^{15}N_{Leaf}$ (positive effects of low means and large plasticities), C_{Shoot} (negative effects of large plasticities) and T_{CV} in SLA (positive effects) and MHR (negative effects). Furthermore, the combined models showed positive effects of large differences (= greater values) in SLA and $\delta^{13}C_{Leaf}$ and negative effects of large differences (= smaller values) in C_{Shoot} on B_{mean}. In contrast, among the predictors selected in

331 the combined model for RY_{mean} were N_{Leaf} (positive effects of high means), SLA (positive
332 effects of high means and low plasticities), $\delta^{15}N_{Leaf}$ (positive effects of high plasticities) as
333 well as C_{Shoot} (negative effects of high variation and large differences to other species). The
334 combined model best explaining variation in RY_{slope} included means in MHR, N_{Leaf}, $\delta^{15}N_{Leaf}$
335 (all positive effects) and $\delta^{13}C_{Leaf}$ (negative effects), plasticity in N_{Leaf} (negative effects) and
336 variation in $\delta^{15}N_{Leaf}$ (positive effects). Furthermore, T_{diff} in SLA and $\delta^{13}C_{Leaf}$ (positive effects)
337 and T_{dist} in N_{Leaf} (negative effects) were incorporated suggesting that species with larger
338 values in SLA and $\delta^{13}C_{Leaf}$ and similar N_{Leaf} compared to neighboring species showed larger
339 increases in RY along the diversity gradient.
340

341 ## Discussion

342 The major goal of this study was to assess the contribution of trait-based metrics beyond
343 species means (T_{mean}) in explaining variation in species performances . These additional
344 metrics were trait plasticities in response to species richness (T_{slope}), trait variation across
345 across communities (T_{CV}) and hierarchical trait differences (T_{diff}) and trait distances (T_{dist}) of
346 focal species to co-occurring species. Our analyses clearly showed that trait means were
347 important in predicting species mean performances and performance plasticities in species
348 mixtures, but trait plasticities as well as the further metrics of trait variation within species
349 improved model predictions. High species means in traits related to plant size and stature,
350 photosynthetic capacity as well as nitrogen acquisition were important for high species
351 performances, whereas it depended on the nature of a trait if the further metrics of tait
352 variation increased or decreased species performances.
353

354 **Average species performances and performance plasticities**

355 Our analyses across five years of the biodiversity experiment showed that average species
356 biomass across mixtures (BM_{mean}) was positively correlated with species RYs (RY_{mean}),

indicating that highly productive mixture species were responsible for positive diversity effects and overyielding of the studied plant communities (Marquard et al. 2009). This impact of highly productive mixture species on overyielding increased with increasing species richness as RY_{mean} correlated positively with the plasticity in RYs along the gradient of plant diversity (RY_{slope}). Overall, 78% of the studied species had $RY_{mean} > 1$ and 58% showed increasing RY at higher species richness (positive RY_{slope}) confirming that many species responded positively to higher plant diversity. However, slopes over diversity for RY were only significant in 10 out of 34 cases for positive relationships and (Fig. A1c) and in 6 out of 25 cases for negative relationships suggesting that in particular positive diversity effects varied greatly with mixture compositions.

Predicting species performances in mixtures with single predictor metrics

Separate analyses of the different trait-based metrics showed that species mean traits (T_{mean}) explained the highest proportions of the variation in mean species performances and performance plasticities (Table 2). Trait differences (T_{diff}), which account for the magnitude and the direction of distinctness in comparison with neighbor species were often higly correlated with T_{mean}. Thus, it is not surprising that both explained a similar portion of variation in species performances and had similar combinations of traits in the separate models for Tmean and Tdiff (Table A4). In contrast, absolute trait distances (T_{dist}) were the least important of the studied metrics in separate models (Table 2). This result emphasizes that not simply the distinctness of a species from others, but its position along a competitive hierarchy is important for high performance, confirming the “trait hierarchy hypothesis”, which is in line with other recent studies (Kunstler et al. 2012, Fort et al. 2014, Kraft et al. 2014, Herben and Goldberg 2014).

Separate models using metrics related to trait plasticity (T_{slope}) explained a larger proportion of variation in species mean performances and performance plasticities than models based on

383 trait variation (T_{CV}) implying that not trait variation per se, but the plastic response to varying
384 growth conditions along the diversity gradient influenced species performances in mixture
385 environments (Table 2).
386
387 **Combining different trait-based metrics to predict species performances**
388 When different groups of trait-based metrics were combined as predictors, at least four out of
389 five were present in the models best explaining mean species performances and performance
390 plasticities. Means species traits (T_{mean}) had always the greatest relative importance, but trait
391 differences (T_{diff}) of the focal species to co-occurring species also achieved high relative
392 importance in the combined models (Fig. 3). Secies biomass values (BM_{mean}) were positively
393 related to high means and larger differences between focal species and neighbors in variables
394 related to plant size and stature. Taller growth compared to neighboring species allows better
395 access to light and may lead to a pre-emption of this resource for smaller species – well
396 known as asymmetric competition — and a superiority of tall-growing species (Gaudet and
397 Keddy 1988, Weiner 1990, Keddy et al. 2002). At the same time, high population-level
398 species biomasses (BM_{mean}) showed negative relationships with means in $\delta^{13}C_{Leaf}$ and were
399 positively related to larger differences in $\delta^{13}C_{Leaf}$ and SLA, two traits which are supposed to
400 correlate with photosynthetic capacity (Dawson et al. 2002). These results underline that
401 several traits related to the acquisition of the same resource contribute to the performance of
402 different species in plant mixtures; if competition for light favors a high performance of tall
403 species, it is still possible that traits that are not related to plant size such as SLA provide
404 opportunities for niche differences and result in a higher performance of smaller-growing
405 species than expected (Maire et al. 2012). Finally, smaller values in $\delta^{15}N_{Leaf}$ and greater
406 differences in N_{Leaf} to co-occurring species also contributed positively to population-level
407 species biomass values (BM_{mean}). Smaller values in $\delta^{15}N_{Leaf}$ and higher N_{Leaf} are typical
408 characteristics of legume species and their dependency on symbiotic N_2 fixation (Gubsch et

409 al. 2011b). The incorporation of these variables is likely due to high population-level species
410 biomass (BM_{mean}) of some legumes (see Fig. A3).
411 The T_{mean} part of combined models best explaining variation in species relative yields
412 (RY_{mean}) was based on high means in SLA and N_{Leaf}. Both, SLA and and N_{Leaf} are key traits
413 of the leaf economic spectrum (Wright et al. 2004) and N_{Leaf} usually also scales positively
414 with rates of photosynthesis (Evans 1989). The incorporation of T_{mean} in these traits supports
415 that fast-growing species with an exploitative strategy of resource acquisition achieve high
416 relative yields in mixtures.
417 The T_{mean} and T_{diff} part of combined models best explaining performance plasticities (RY_{slope}),
418 however, included similar predictors as the BM_{mean} model, probably due to the fact that
419 species with high population-level species biomass (BM_{mean}) also increased their performance
420 (positive RY slopes) in the mixtures (Fig. 2b, A3). In contrast to the BM_{mean} model, the T_{mean}
421 part of the RY_{slope} model also included positive effects of $\delta^{15}N_{Leaf}$ and N_{Leaf}. While high
422 means in N_{Leaf} could indicate a high performance plasticity of legumes, high means in $\delta^{15}N_{Leaf}$
423 are more typical for non-legume species. In addition, the T_{dist} part suggested that smaller
424 differences to co-occurring species in N_{Leaf} were related to high performance plasticity in the
425 mixtures, while T_{slope} for N_{leaf} and T_{CV} for $\delta^{15}N_{Leaf}$ indicated that low within-species variation
426 in nitrogen-related traits were related to increasing RY_{slope} values in the mixtures. If
427 increasing facilitative interaction between legumes and non-legumes at higher plant diversity
428 would be a major driver of increasing species performances in the mixtures, a negative
429 relationship between RY_{slope} and T_{slope} would have been expected for $\delta^{15}N_{Leaf}$, because
430 increased provision of legume-derived ^{15}N-depleted nitrogen at higher plant diversity would
431 result in negative T_{slope} values. Overall, T_{slope}, T_{CV} and T_{dist} had a small relative importance in
432 the combined models for RY_{slope} (Fig. 3). Therefore, our results more likely support that
433 legumes as well as non-legumes were among the species with the greatest positive
434 performance plasticity in the mixtures (Fig. A3c).

435 In contrast to the RY_{slope} model, trait plasticity (T_{slope}) had a greater relative importance in the
436 models for species mean performances (B_{mean}, RY_{mean}) and T_{CV} also achieved a higher
437 relative performance in the combined BM_{mean} model (Fig. 3). Dependent on the considered
438 trait, T_{slope} showed positive or negative relationships to species performance supporting the
439 view that high trait variation has not necessarily an adaptive value in the mixtures (Valladares
440 et al. 2007). For example, greater slopes and larger variation in traits related to plant stature
441 and structural stability (C_{Shoot}, MHR) showed negative relationships to mean species
442 performances, suggesting that the high variation in these traits was related to passive growth
443 responses rather than adaptive plasticity. In addition, T_{slope} in SLA showed a negative
444 relationship with RY_{mean}. Variation in SLA is a well-known response to changes in light and
445 nutrient availability (Evans and Poorter 2001, Hodgson et al. 2011). Studies on trait variation
446 in the Jena Experiment have shown that the formation of leaves with higher SLA is due to a
447 greater canopy density of more diverse plant communities (Roscher et al. 2011). The negative
448 relationship with RY_{mean} emphasizes that plasticitiy in SLA does not have an adaptive values
449 to increase species-level performance in mixtures compared with monocultures. In contrast to
450 these traits, T_{slope} values for leaf stable isotopes ($\delta^{15}N_{Leaf}$, $\delta^{13}C_{Leaf}$) were positively related
451 with species mean performances, suggesting that plasticity in physiological traits might have
452 an adaptive value.
453
454 Although our combined models increased the predictability of species performances, the
455 choice of traits in our analysis was restricted to aboveground plant traits related to the
456 acquisition of light and nutrient, which had been measured at one time and therefore only
457 incorporated a certain proportion of trait variation within and between species. Furthermore, it
458 is likely that further traits related to root characteristics and life cycles also explain variation
459 in species performances and should be considered in future studies. Nevertheless, our results
460 show that different metrics beyond mean species traits, namely trait plasticities and trait

differences to co-occurring species are important in explaining the mean performance and the performance plasticity of 59 studied grassland species in the Jena Experiment. The incorporation of different traits in the varying metrics emphasizes that the operating mechanisms are trait-dependent. These results are in line with several recent studies focusing on the outcome of pairwise species interactions (Kraft et al. 2014, Fort et al. 2014) and add that similar mechanism are valid in multi-species mixtures. Taken together, we conclude that the inclusion of trait variation as well as the disparity among co-occurring species are promising avenues in extending trait-based analyses to understand community assembly and the contribution of individual species in structuring plant communities.

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554 Table 1: Summary of functional traits derived from trait measurements

Trait	Abbreviation	Unit	Description
Shoot length	L_{Shoot}	cm	stretched shoot length
Mass:height ratio	MHR	$mg_{shoot}\ cm^{-1}{}_{shoot}$	shoot dry mass per unit length
Shoot carbon concentration	C_{Shoot}	$mg\ C\ g_{shoot}^{-1}$	shoot carbon concentration
Specific leaf area	SLA	$mm^2{}_{leaf}\ mg^{-1}{}_{leaf}$	leaf area per leaf dry mass
Leaf $\delta^{13}C$	$\delta^{13}C_{Leaf}$	‰	^{13}C isotopic signature of leaves
Leaf nitrogen concentration	N_{Leaf}	$mg\ N\ g_{leaf}^{-1}$	leaf nitrogen concentration
Leaf $\delta^{15}N$	$\delta^{15}N_{Leaf}$	‰	^{15}N isotopic signature of leaves

555

Table 2: Summary of the best statistical model predicting population-level species biomass (BM_{mean}), relative yields (RY_{mean}) and their plasticities in response to the plant diversity gradient (RY_{slope}) from different trait-based metrics (T_{mean}, T_{slope}, T_{CV}, T_{diff}, T_{dist}) separately. See Table 1 for abbreviations of trait names.

	AIC	R^2	Intercept	L_{Shoot}	MHR	C_{Shoot}	SLA	$\delta^{13}C_{Leaf}$	N_{Leaf}	$\delta^{15}N_{Leaf}$
BM_{mean}										
T_{mean}	222.18	0.326	4.988	0.768	0.480	-0.581			0.884	
T_{slope}	226.98	0.269	4.988		0.427	-0.402		0.508		0.370
T_{CV}	232.88	0.106	4.988		-0.574					
T_{diff}	226.28	0.278	4.988	0.559		-0.669		0.505	0.765	
T_{dist}	236.63	0.047	4.988	0.382						
RY_{mean}										
T_{mean}	211.69	0.216	1.041			-0.551	0.417		0.635	
T_{slope}	217.78	0.100	1.041	0.504				0.315		
T_{CV}	220.64	0.055	1.041	0.369	-0.382					
T_{diff}	211.83	0.214	1.041			-0.551	0.403		0.623	
T_{dist}	219.86	0.036	1.041				0.288			
RY_{slope}										
T_{mean}	25.16	0.267	0.046	0.130	0.084	-0.103	0.080		0.120	
T_{slope}	30.61	0.110	0.046		0.080					0.067
T_{CV}	30.92	0.075	0.046		-0.086					
T_{diff}	31.86	0.122	0.046	0.090	0.073		0.082			
T_{dist}	32.97	0.042	0.046	0.064						

Shown are standardized regression coefficients for each predictor variable included in the best multiple regression models together with AIC and R^2 statistics. The best five models for each analysis are summarized in the Supplementary Material Appendix Table A4.

Table 3: Summary of the best five statistical models predicting population-level species biomass (BM_{mean}), relative yields (RY_{mean}) and plasticities in response to the plant diversity gradient (RY_{slope}) from different trait-based metrics (T_{mean}, T_{slope}, T_{CV}, T_{diff}, T_{dist}) in combination. See Table 1 for abbreviations of trait names.

	BM_{mean}					**RY_{mean}**					**RY_{slope}**				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
AIC	212.99	213.11	213.28	213.39	213.46	206.99	207.07	207.13	207.33	207.43	12.89	13.07	13.27	13.65	13.68
R^2	0.496	0.544	0.494	0.526	0.376	0.367	0.367	0.344	0.364	0.341	0.480	0.496	0.459	0.491	0.480
Intercept	4.988	4.988	4.988	4.988	4.988	1.041	1.041	1.041	1.041	1.041	0.046	0.046	0.046	0.046	0.046
T_{mean}															
L_{Shoot}		0.618	0.434	0.682	0.760										
MHR		0.537		0.450	0.455						0.160	0.179	0.152	0.140	0.169
SLA						0.564		0.673	0.630						
$\delta^{13}C_{Leaf}$	-1.797	-1.041	-1.668	-1.171	-0.864						-0.465	-0.470	-0.450	-0.486	-0.455
N_{Leaf}							0.552	0.566	0.504	0.629	0.178	0.172	0.148	0.154	0.146
$\delta^{15}N_{Leaf}$		-0.401		-0.381	-0.565						0.197	0.207	0.148	0.193	0.157
T_{slope}															
L_{Shoot}						0.293									
MHR	0.482		0.497											0.041	
C_{Shoot}		-0.559		-0.591	-0.520							-0.046			-0.044
SLA						-0.412	-0.335	-0.310	-0.356	-0.277					
$\delta^{13}C_{Leaf}$		0.403		0.472	0.358										
N_{Leaf}											-0.057	-0.059		-0.055	
$\delta^{15}N_{Leaf}$		0.534		0.588	0.552	0.392	0.488	0.438	0.483	0.433					
T_{CV}															

MHR	-0.373	-0.318	-0.365		-0.394										
SLA	0.488	0.491	0.485	0.412	0.552										
C_{Shoot}						-0.426	-0.453	-0.435	-0.498	-0.374					
$\delta^{15}N_{Leaf}$											-0.168	-0.185	-0.135	-0.163	-0.150
T_{diff}															
L_{Shoot}	0.437														
C_{Shoot}	-0.594		-0.573		-0.285	-0.889	-0.855	-0.845	-0.891	-0.799					
SLA		0.531		0.562	0.526		0.603			0.635	0.114	0.127	0.102	0.112	0.115
$\delta^{13}C_{Leaf}$	2.108	1.381	1.981	1.527	1.260						0.451	0.447	0.450	0.462	0.446
N_{Leaf}	0.530		0.517			0.611									
T_{dist}															
SLA							0.280		0.247						
N_{Leaf}											-0.095	-0.083	-0.089	-0.086	-0.077

Shown are standardized regression coefficients for each predictor variable included in the best multiple regression model together with AIC and R^2 statistics.

568 Figures legends

569

570 Figure 1: Overview of trait-based metrics used for predicting mean species performances and
571 performance plasticities in mixtures. For simplicity, only two species (A and B) are displayed
572 for (a) trait means (T_{mean}), (b) trait plasticity (T_{slope}), (c) trait variations (T_{CV}), (d) the concept
573 of calculating for each species in every mixture hierarchical and absolute trait differences to
574 co-occurring species according to Kraft et al. (2014), which were averaged across
575 communities to derive (e) trait differences (T_{diff}), and (f) trait distances (T_{dist}). Note that
576 circular symbols (a-c) depict trait values of a particular species in a mixture, while open
577 triangles (e) show the mean difference of the trait values of the focal species (A or B) to co-
578 occurring species in a mixture, and closed triangles (f) show means of the absolute difference
579 of trait values of the focal species (A or B) to co-occurring species in a mixture.

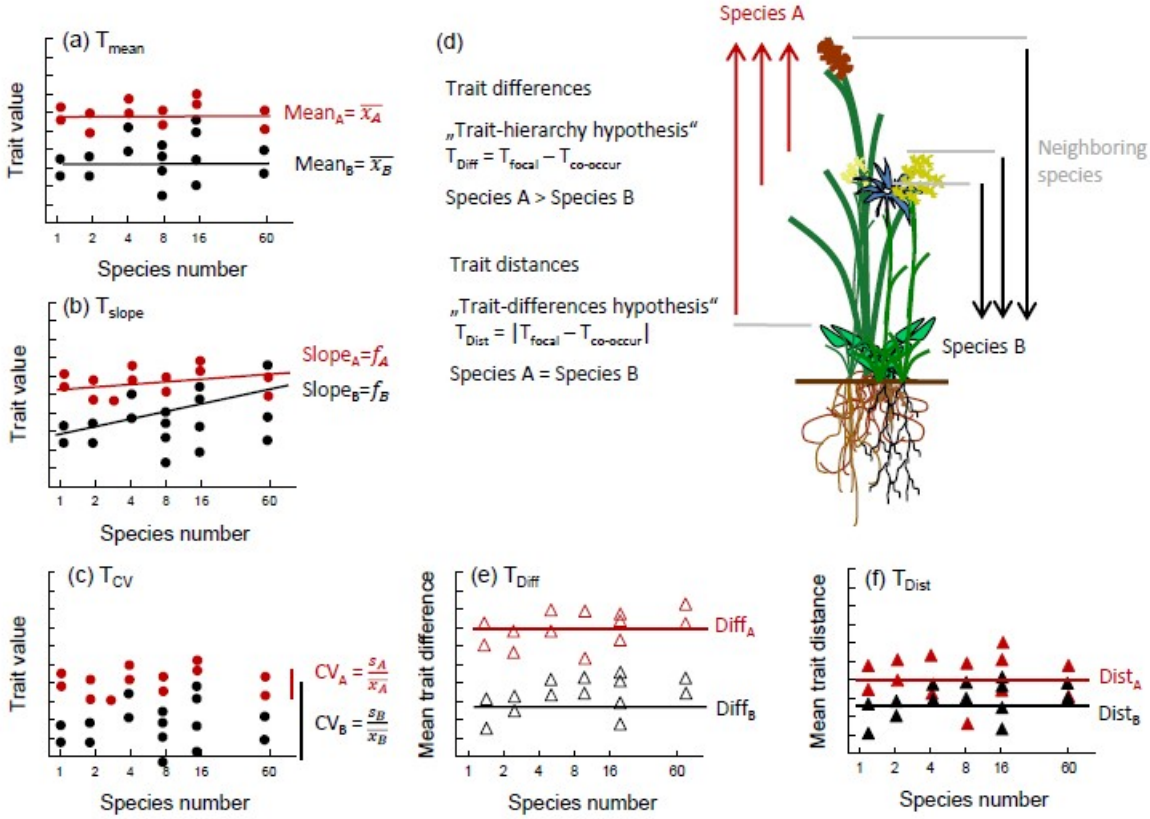
580

581 Figure 2: Population-level relative yields (RY_{mean}, a) and relative yield plasticities in response
582 to the plant diversity gradient (RY_{slope}, b) of species plotted against their population-level
583 biomasses (BM_{mean}) in mixture and relative yield plasticities (RY_{slope}) plotted against RY_{mean}
584 (c). Pearson correlation coefficients (r) between variables and their significances (p) are listed
585 within the plots.

586

587 Figure 3: Summary of regression analyses for z-transformed values of population-level
588 biomass (BM_{mean}) in mixture, relative yields (RY_{mean}) and their plasticities in response to the
589 plant diversity gradient (RY_{slope}) of the different species as functions of z-transformed values
590 of trait-based metrics (T_{mean}, T_{slope}, T_{CV}, T_{diff}, T_{dist}). Shown is the relative importance of the
591 different groups of predictors averaged across the best five models as the R^2 contribution
592 averaged over orderings among regressors (Grömping 2006).

593 Figure 1



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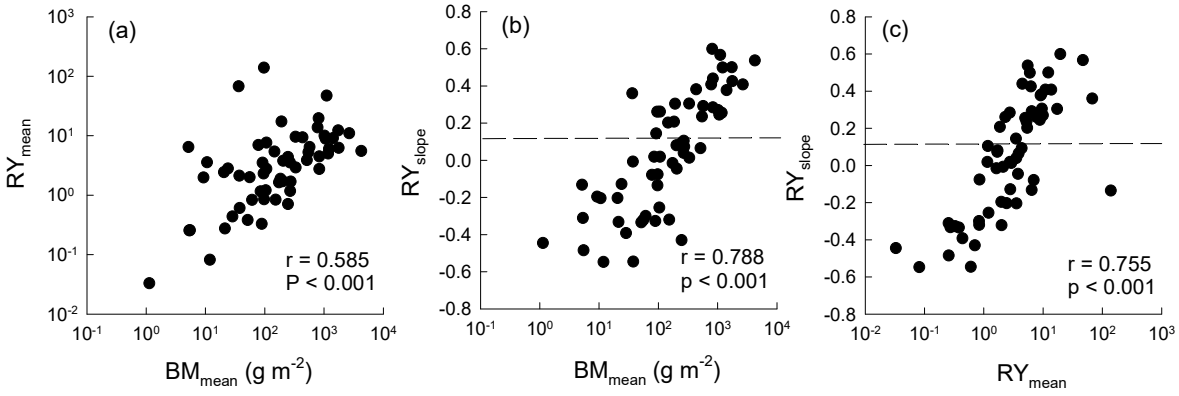
595 Figure 2



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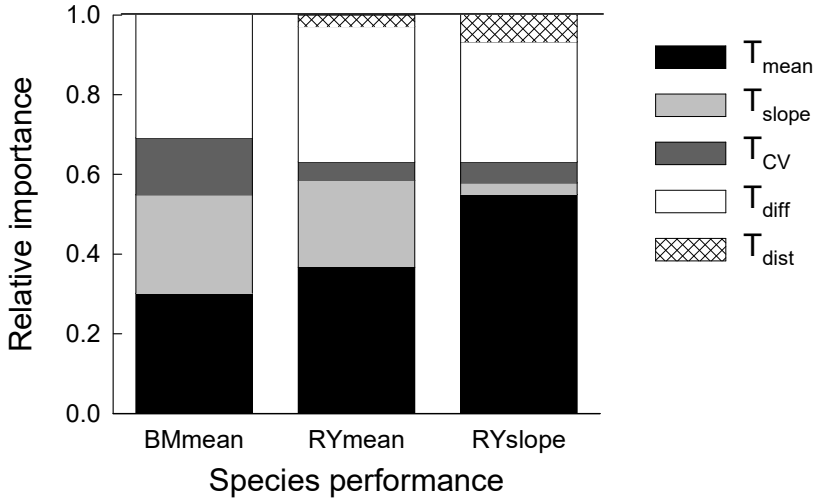
598 Figure 3



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